RESEARCH ARTICLE

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INCREASING SERUM LEVEL OF TUMOR NECROSIS FACTOR ALPHA IN SOME GASTROINTESTINAL CANCERS IN IRAQI PATIENTS

ABSTRACT:
Gastrointestinal cancers (GITs) are a worldwide problem particularly in the highly developed countries. In Iraq, gastric cancer (GC) is the 9th most common cancer while colorectal cancers (CRC) is considered as the 7th most common cancer among all cancer patients in both males and females. The objective of this study was to estimate the serum level of Tumor Necrosis factor alpha (TNF-α) in some Iraqi colorectal and gastric cancer patients. In this study, 54 serum samples were collected starting from the 1st of January till the mid of March 2011 to investigate the TNF-α serum level by using ELISA technique. Thirty-eight samples were gastric (H. pylori +ve) and colorectal cancer patients (GC=17, CRC=21) and 16 samples considered as a healthy control group. The results showed that TNF-α serum levels of both GIT tumors were increased significantly (P<0.05) comparing to the healthy control group. In conclusion, as previous literature showed a correlation between the increase of TNF-α production and the genetic expression of the TNF-α alleles, the present data recommend further analysis of TNF-α alleles for Iraqi GC patients. This could be useful to detect the risk of failure of first-line chemotherapy and overall survival of Iraqi GC patients. Furthermore, since high serum levels of TNF-α in CRC patients was shown previously to correlate with worse prognosis of the tumors, the present data could point out to use this elevation as a biomarker for tumor prognosis in Iraqi CRC patients.

KEY WORDS:
TNFα, Colorectal Cancer, Gastric Cancer.

INTRODUCTION:
Gastrointestinal cancers (GITs) are a worldwide problem. For example: about 4,500 to 6,000 new cases are registered in the United States each year. Gastric cancer (GC) is the 4th most common cancer in the world; it is more common in men and causes about 800,000 deaths worldwide every year (Buckland et al., 2009). In Iraq the GC is the 9th most common cancer among all cancer patients (Iraqi Cancer Board, 2008). On the other hand, colorectal cancers (CRC) are considered as the 1st most common and aggressive type of cancer worldwide. In 2006 there were about 412900 new CRC cases in Europe and 142672 in the United States (Cutsem et al., 2008). In Iraq CRC is considered as the 7th most common cancer type in both males and females (Iraqi Cancer Board, 2008).

The systemic and local cytokine microenvironment resulted from GITs cancers were shown to modulate the immunogenicity and to affect the anti-tumor immune function of the tumor-infiltrating lymphocytes. This has generated evidence that individual pro-inflammatory cytokine and anti-inflammatory cytokines may have a complex role in gastrointestinal carcinogenesis (Dalerba et al., 2003). TNF-α is a potent pro-inflammatory, multifunctional cytokine which...
plays a key role in apoptosis and in cell survival, as well as in inflammation and immunity. By contrast, it has conflicting roles in cancer, as a necrotic and promoting/growth factor (Asher et al., 1987). The role of TNF-α in CRC has been shown to modulate epithelial-to-mesenchymal transition (EMT) (Asher et al., 1987; Bates and Mercurio, 2003), while in GC it is a mediator of the immune response to H. pylori and shares many biological activities with interleukin -1(IL-1) (Bates and Mercurio, 2003).

The aim of the present study is to estimate the serum level of TNF-α in GC and CRC patients and discussing the data with previous international findings to be applied for Iraqi patients.

MATERIAL AND METHODS:
Sample collection:
Samples from 38 GC (H. pylori +ve) and CRC patients (GC = 17, CRC = 21) and 16 healthy individuals were collected (after definitive diagnosis and before taking the chemotherapy) at the Oncology clinic / Baghdad Teaching Hospital and at the Teaching Hospital for the GIT and liver diseases /Medical city directory starting from the 1st of January till the mid of March 2011. A questionnaire was made to obtain the demographic data such as name, address, sex, ABO, Rh, tobacco smoking, Alcohol consumption, food type and family history, while the histopathological data like cancer type, staging and tumor cell differentiation were taken from the patient’s files. Five to ten ml of venous blood were drown from each cancer patient before centrifugation at 4°C to obtain serum used to detect TNF-α levels by ELISA technique.

Detection of TNF-α by ELISA:
ELISA kit (Cell Singling Technology, Denver, MA, USA), was used according to the manufacturer's instructions. Briefly, the microtiter plate was pre-coated with an antibody specific to TNF-α then standards and samples were added to the appropriate microtiter plate wells. A biotin conjugated antibody preparation specific for TNF-α and avidin conjugated to Horseradish peroxidase(HRP) were added to each well. After incubation, 3,3',5,5'tetramethyl-benzidine (TMB) substrate solution was added to all wells. Only those wells that contain TNF-α biotin-conjugated antibody avidin exhibited a change in color. The enzyme substrate reaction was terminated by adding of (according to the manufacturer’s instructions), 3 M sulphuric acid solution, then the color change was measured spectrophotometrically (ASYS, Australia) at a wavelength 450 nm ± 2 nm. Finally, TNF-α concentration was determined by comparing the optical density (O.D.) of each sample to the standard curve.

Statistical Analysis:
Statistical analysis was performed using Student’s T-test (Microsoft office Excel worksheet, Microsoft Company, USA). Data were considered significant at P<0.05.

RESULTS:
Demographic and Histopathological Data:
The demographic data showed that the mean age of the patients was 55.6 years ,their sexes were 16 males (42%) and 22 females (58%), their ABO system was categorized as the falling : A 12 (31%),B 10 (27%), AB 6 (15%) and O 10 (27%), while the Rh factor was positive in 8 (22%) and negative in 30 (78%) (Fig. 1).

Fig. 1. Sex, ABO system, and Rh data

Non tobacco smoker patients were 22 (57%), mild tobacco smokers were 2(5%), and heavily tobacco smokers were 14 (38%). Alcohol consumers were 4(10%) and non-alcoholic ones were 34 (90%). Vegetarian patients were 4 (10%), meat eating patients were 1 (3%), and patients consumed mixed diet were 33 (87%). The family history was positive in 17 (45%). Patients who have relatives suffer from GIT cancers were 7 (41%) while the rest 10 (59%) have relatives suffer from other organs cancers (Fig. 2).

Fig. 2. Data of tobacco smoking, alcohol consumption, food intake, and family history of the GIT cancers patients.

The histopathological data showed that the cancer types were adenocarcinomas 36 (95%) and others 2 (5%) were diagnosed as signet ring and mucinous carcinomas (Fig. 3). The tumor cell differentiation was classified as well differentiated 1 (2%), moderately
differentiated 20 (52%) and poorly differentiated 17 (46%).

![Cancer Type Diagram]

**Fig. 3.** Histological cancer types in GIT cancers patients.

**Serum levels of TNF-α:**

The results showed that serum TNF-α levels of both GIT tumors increased significantly (P<0.05) comparing with the healthy control group. Corresponding figures were 989.3 ± 67.5 SE in GIT cancers patients and 528 ± 98.3 SE in healthy controls (Fig. 4).

![Serum TNF-α Levels Graph]

**Fig. 4.** Serum TNF-α levels of GIT cancer patients

* Significant at p<0.05

**DISCUSSION:**

A number of studies attempted to establish a link between inflammation and carcinogenesis, including experiments to assess the ability of pro-inflammatory cytokines such as TNF-α, to induce tumors. TNF-α is a cytokine that is produced early in the inflammatory cascade and has been shown to promote carcinogenesis in multiple types of both human and animal tumors like murine skin tumors (Moore et al., 1999; Suganuma et al., 1999). TNF-α was shown to promote carcinogenesis by up-regulating Nuclear Factor-kappa B (NF-kb) leading to up-regulation of other factors that cause cell proliferation and morphogenesis leading to cancer formation in the GIT (Varella et al., 2001; Brantjes et al., 2002). TNF-α does not only work as a carcinogen but also has a significant role in cancer metastasis (Cubillos et al., 1997), neovascularisation and angiogenesis (Shin et al., 2000), cancer cells detachment from the primary site (Nozawa et al., 2000), and increased tumor cell motility (Condeelis et al., 2001; Kassis et al., 2001; Price and Collard, 2001). TNF-α also increases invasion of the extra cellular matrix (Hajitou et al., 2001), and facilitates the entry of tumor cells into vasculature and lymphatics (Simiantonaki et al., 2002). At last, TNFα may help in the proliferation of metastasized tumor cells (Tanaka et al., 1999; Hideshima et al., 2001).

In the present study, the significant increase of serum TNF-α level of the H. pylori +ve gastric tumors of Iraqi patients is in line with previous results which demonstrated that serum TNF-α level was elevated in patients with H. pylori infection (Guiraldes et al., 2001). Overexpression of this cytokine due to this pathogen was also involved in tumor induction and in promotion of stomach cancer (Sun et al., 2006; Senthilkumar et al., 2010). Moreover, some previous data indicated that H. pylori gene products have a TNF-α inducing activity and act as tumor promoters during GC carcinogenesis. These are TNF-α inducing protein (Tipa) gene family in H. pylori genome. For example Tipa and HP-MP1 gene products act as new H. pylori mediated carcinogenic factors through strong induction of TNF-α gene expression and NF-kb activation with down regulation of Inhibitory kb (Ikb) (Suganuma et al., 2001; Waterston and Bower, 2004; Suganuma et al., 2005). Previously, it was shown that the presence of the TNF-α allele involved in gene transcription was associated with higher plasma levels of TNF-α at the time of tumor diagnosis (Suganuma et al., 2005). Expression of the two alleles associated with increased TNF-α production were found to be a risk factor for failure of first-line chemotherapy, a shorter progression-free survival and a reduction in overall survival (Suganuma et al., 2006).

The present elevation of serum TNF-α levels in CRC patients was also reported in previous work in patients from different countries other than Iraq (Ardizzoia et al., 1992; Belluco et al., 2000; Kaminska et al., 2005; Nikiteas et al., 2005). This was correlated with worse prognosis (Ueda et al., 1994; Nakashima et al., 1998; Roselli et al., 2003) and its role has been linked to all steps involved in cancer initiation, promotion and progression including cellular proliferation and transformation, invasion, angiogenesis, metastasis and survival in CRC and other malignancies (Etoh et al., 2000; Suzuki et al., 2001; Guadagni et al., 2007).

The underlying mechanisms involved in increased TNF-α in cancer is still debated. It is well known that many tumors, including CRC, produce various inflammatory cytokines (Stattin et al., 2003). Among them TNF-α,
frequently detected in biopsies from human cancer, produced either by epithelial tumor cells or stromal cells (Roselli et al., 2003) and its production has been associated with poor prognosis, loss of hormone responsiveness and cachexia/asthenia (Szlosarek and Balkwill, 2003; Tisdale, 2008). Also, it has been noticed that in patients with CRC, TNF-α and its mRNA could be detected in relatively high amounts in macrophages at the site of the tumor which could be another source of serum elevation of the corresponding protein (Beissert et al., 1989).

In conclusion, the present data showed high serum level of TNF-α in GC patients which may be due to the effect of TNF-α inducing protein (Tippa). On the other hand, this elevation in Iraqi GC patients could be used as a useful biomarker for tumor prognosis in Iraq. It was established that the elevation of TNF-α in CRC patients may be due to the tumor infiltrated macrophages production of TNF-α. Therefore, further studies are recommended to detect the correlation between elevated sera TNF-α and the mRNA expression of TNF-α gene alleles to explore its role in the first-line chemotherapy and survival rates in Iraqi CRC patients.

REFERENCES:


في مصل مرضى بعض سرطانات الجهاز الهضمي Tumor Necrosis Factor Alpha في العراق

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تعد سرطانات الجهاز الهضمي من أكثر المشكلات الصحية في العالم خاصة في البلدان المتقدمة أو ما يسمى بلدان العالم الأول. يقع سرطان المعدة في العراق بالترتيب الثاني والثالث والدفاع والوقاية والمستقيم بالترتيب السابع ضمن قائمة العشر سرطانات الأكثر انتشارا بين الرجال والنساء المصابين بمرض السرطان. كان الهدف من هذه الدراسة هو قياس مستوى TNF-α في المرضى الذين يعانون من سرطان المعدة والقولون والمستقيم. وقد جمعت 54 عينة من المرضى.

تم قياس TNF-α في المرضى الذين يعانون من سرطان المعدة والقولون والمستقيم. وتم تجميع عينات من المرضى الذين يعانون من سرطان المعدة والقولون والمستقيم. وتم تجميع عينات من المرضى الذين يعانون من سرطان المعدة والقولون والمستقيم.

المكونات:

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